Azolla-Microbial Consortia for Enhancing Milk Quality And Milk Production in Milch Animal

PhD Synopsis submitted to

Swami Ramanand Teerth Marathwada University, Nanded

For the research work leading to the award of the degree of

Doctor of Philosophy
In Microbiology
Under the faculty of Science and Technology

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2024

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INTRODUCTION:

Milk is a highly nutritious food that plays a crucial role in human health and development. It as various significance it includes Milk contains essential nutrients like calcium, protein, vitamins (A, D, B12), and minerals (potassium, phosphorus) necessary for growth and development. The high calcium content in milk supports strong bones and teeth, reducing the risk of osteoporosis. Milk provides high-quality proteins, which are important for muscle repair and aintenance. Milk is a source of immunoglobulins and beneficial compounds that enhance the immune system. Being about 87% water, milk helps maintain hydration while also providing nutrients. Milk is a base for many dairy products like cheese, butter, yogurt, and cream, contributing to diverse culinary applications. With carbohydrates, fats, and proteins, milk is an energy-dense food ideal for growing children, athletes, and individuals recovering from illness. Milk plays an integral role in many cultural traditions and is a livelihood source for millions engaged in dairy farming.

The gut microbiota carried out its role in several biological processes across different species, and which helps in dairy cow milk production and quality is of interest to the dairy industry and the wellbeing of the rural economy. Microbial products help in food fermentation, methane and nitrogen emissions, and fiber breakdown. *Bifdobacterium*, *Ruminococcus*, and *Fibrobacter* help in cellulose digestion, whereas *Succino vibrinonaceae* impacts methane emissions. As a result of fermentation Volatile fatty acids compounds formed by microbes in the gut of ruminants that provide an energy source to the host. For example, *Clostridium sticklandii* is involved in NH3 production.(Faheem Ahmed Khan.*et.al*.2023).

The gut of dairy cattle consists of majority of bacteria which secreted important enzymes, which play a primary role in plant degradation. Multiple glycoside hydrolases work to deconstruct the intricate chemical structure of plant biomass. Lipases can regulate fatty acid metabolism in the rumen and control of lipolysis in the rumen could play a vital role in limiting the biohydrogenation of polyunsaturated fatty acids. Xylanase breaks down the polysaccharide β-1,4-xylan, a main chain of hemicellulose, while carboxymethyl cellulase (CMCase) attacks low-crystalline regions in the cellulose fiber, creating free chain ends by degrading β-1,4-glucan. Dehydrogenase, urease, and protease can react with protein and urea, which further supplies ideal protein nutrients to the host. Meanwhile, the rumen microbiota plays a crucial role in digesting the feed into volatile fatty acids (VFA), ammonia, and

microbial crude protein (MCP), which can further supply nutrients for ruminants. (Yangyi Hao.*et.al*,2021.)

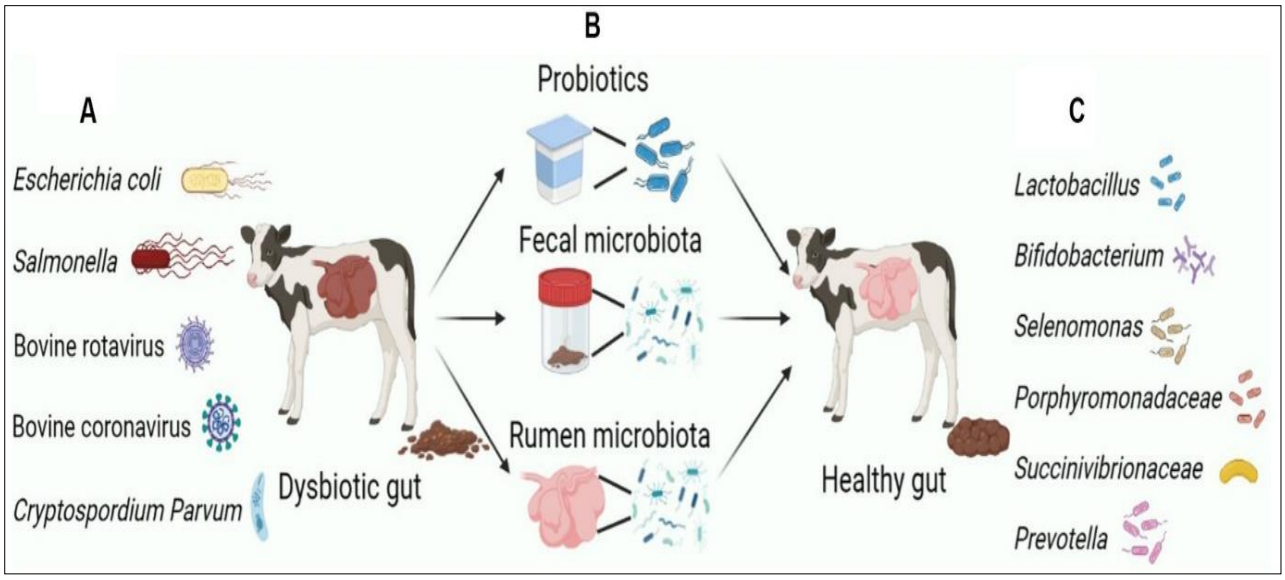

Fig: Involvement of Microorganisms in Milch animals

India is well known for its biological diversity and largest livestock population in the world. For fulfilment of nutrition demand the alternate sources of good quality unconventional feed for efficient livestock production is needed. For alternatives to feed to different species of animals, azolla is a great option, as a sustainable feed for livestock. It consist of most important nutrients which are required for all classes of including dairy, poultry and fish (Basak B.*et.al*.2002. Feeding with azolla increase the milk production in cow and improves the weight of broiler chicken and increase the egg production. In birds, feed with extra supplemented azolla showed extra weight gain. It is also used as human food, animal feed, in biofuel production, water purifier, weed control, in phytoremediation therefore referred as 'green gold mine'. (Sonu KumarYadav 2023).

Azolla is a free-floating aquatic fern. There are six species of Azolla such as Azolla Carolina, Azolla nilotica, Azolla filiculoids, Azolla Mexicana, Azolla microphylla and Azolla pinnata. The Azolla Pinnata is a common species found in India. The previous investigation showed the Azolla microphylla is a suitable species for quick biomass production under tropical and sub-tropical climate condition. Azolla filuculods is temperate species can be grown under temperate region. Now-a-days a cross between Azolla microphylla and Azolla filiculoids are also available in India (Kalyani.W.B).

A botanical classification of Azolla as,

Kingdom	:	Plantae
Clade	:	Tracheophytes
Division	:	Polypodiophyta
Class	:	Polypodiopsida
Order	:	Salviniales
Family	:	Salviniaceae
Genus	:	Azolla

Azolla are spore-producing (heterosporous), free- floating fresh water vascular plants that have neither seeds nor flowers. It is found in quiet waters, ponds, ditches, canals and paddy fields. Azolla live symbiotically with Anabaena Azolla, nitrogen-fixing blue-green algae. The endosymbiont, which is nitrogen-fixing, provides sufficient nitrogen for both itself and its host (Azolla). The Azolla fern, on the other hand, provides a protected environment for the alga and also supplies it with a fixed carbon source. Because of this symbiotic presence of nitrogen-fixing cyanobacterium with Azolla, and its tendency to block out light to prevent any competition from other plants, it is used in rice cultivation as companion plant.(Nevine M.*et.al*.2017).

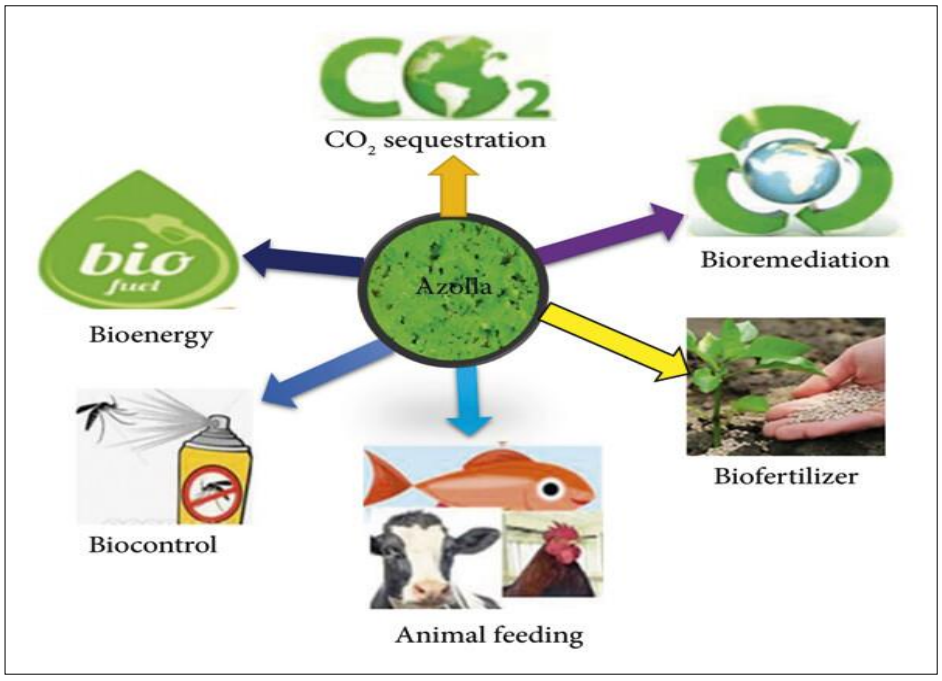

Fig: Applications of Azolla.

Azolla fern is rich in micro-nutrients and non-enzymatic anti-oxidants. The nutritional profiling of Azolla revealed that the fern is rich in non-enzymatic antioxidants and micro-

nutrients. Antioxidant properties of Azolla caroliniana eclipsed the properties of Azolla microphylla. However, Beta-carotene content of Azolla caroliniana overshadowed Azolla microphylla illustrating its better anti-cancerous and cardio protective properties. Broadly, the micro–nutrient profile of Azolla microphylla is higher than Azolla caroliniana. Azolla microphylla is rich in essential amino acids including Histidine, Lysine, Methionine and Valine. Quantitative analysis of B-vitamins showed that Azolla microphylla is rich in Vit B12 and Vit B9 than Azolla caroliniana (Mohd Azhar.et al.June 2018).

Due to the scarcity of concentrate and green fodder and its high prices in animal feeding, the quest for readily available and inexpensive alternative sources has become critical for breeders seeking to minimize the cost of animal production. Azolla is an invasive plant that grows faster in freshwater lakes, rivers, wetlands, and ditches in both temperate and tropical countries by a few precautionary steps with low production costs. It can modify biodiversity and aquatic ecosystems substantially. Azolla has a symbiotic relationship with the nitrogen-fixing blue-green alga Anabaena azollae, which increases the protein content of Azolla, making it one of the greatest alternative feed ingredients as a source of protein, with 25–30% protein, 7–10% amino acids, vitamins (Beta Carotene, vitamin A, vitamin B12), minerals (calcium, potassium, phosphorus, ferrous, magnesium, copper, etc.), and antioxidants. Several studies showed that Azolla can be fed to animals like cow, buffalo, sheep, goat and rabbit because it is easily digestible (because to its high protein and low lignin content), increases feed efficiency, average daily gain of animals, and milk production by 15–20% (Soad EI Naggar.2022) .

Azolla develops a symbiotic relationship with blue green algae, Anabaena azollae. An azolla plant is a fern frond consisting of a main stem growing at the surface of the water, with alternate leaves and adventitious roots at regular intervals along the stem. Secondary stems develop at the axil of certain leaves. Azolla fronds are triangular or polygonal and float on the water surface individually or in mats. Plant diameter ranges from 1inch (1–2.5 cm) for small species, such as Azolla pinnata, to 6 inches (15 cm) or more for A. nilotica. A shallow fresh-water pond, similar to the environment found in a taro lo'i, is the ideal environment for azolla. When taro reaches maturity, it shades out the azolla below the canopy, gradually killing it and resulting in the release of nutrients into the soil-water system, where they become available for uptake by the taro. Azolla well grow in water or wet mud, and it dies within a few hours under dry conditions. Azolla can survive a water pH range of 3.5–10, but optimum growth occurs between pH 4.5 and 7 and the optimum temperature is between 64 and 820 F (18–280C). The growth rate gradually declines as salinity increases. Azolla grows

in full to partial shade (100–50% sunlight), with growth decreasing quickly under heavy shade. Azolla is established by vegetative propagation (Abhay Kumar.et.al.2018).

Plants and microorganisms form complex associations displaying diverse interactions ranging from mutualism to pathogenicity. The habitat for microorganisms can be both the interior (endosphere occupied by endophytes) and the surroundings (phyllosphere, rhizoplane, and rhizosphere occupied by epiphytes) of the host plant (Yan-Qiu Yang.*et.al*.2023)

The azolla symbiotically associated with anabaena in which two partners have successful co-evolved into system that makes important contribution to ecology, biofertilization and biotechnology therefore term known as superorganism. (The azolla foundation.)

Applications of azolla:

- Livestock feed
- Production of biofuel
- Bio--fertilizer
- Green manure
- Bioremediation
- Human food
- Mosquito repellent
- Carbon sequester of CO_2

REVIEW OF LITERATURE:

Aquatic plant, free floating fern Azolla which belongs to the family Azollaceae is a good source of protein and it contains essential amino acids, minerals such as iron, calcium, magnesium, potassium, phosphorus, manganese and vitamin A ,precursor beta-carotene and vitamin B12. Azolla pinnata being used as unconventional feed and protein supplement for animals like ruminants, pigs, poultry and fish. It is also found to contain probiotics and biopolymers. It has been also known as “green gold mine” because of its high nutritive value. Azolla appears to be a potential source of protein and has a considerably high feeding value. It is easy cultivate, high nutritive value and gives high productivity (Tadavi FR, Patil KB and Kadam RL.2023).

Azolla is free floating water fern symbiotically associated blue-green algae which is responsible for nitrogen fixation. Azolla has triangular or polygonal fronds (leaves) and float on water surface. For the cultivation of azolla, a shallow fresh water pond ideal which differ in size according to quantity of feed. For small holders an area of 6 × 4 feet is sufficient to produce about one kg of supplemental feed per day throughout the year. Sieved fertile soil mixed with cow dung and water uniformly spread in the pond. Biogas slurry was also used instead of cow dung. The harvested rain water in rainy season also used for cultivation of azolla, it will ensure faster and excellent growth of azolla. (K.Giridhar *et.al 2012*).

In India large livestock demand is there. For fulfilment of nutrient demand and for production good quality feed, azolla is great option for sustainable feed for livestock. In dairy cows, feeding of azolla can increase milk production by 15 to 20 percent and in poultry, it improves the weight of broiler chicken and increases the egg production in layers. The birds that received normal feed with 5% extra supplementation of azolla showed a 10 to 12% increase in the total body weight. Dietary inclusion of sun-dried azolla up to about 5% levels had no adverse effect on the production performance of broiler chicken and reduces the feeding cost making the net profit per bird being higher. Pit is dung on ground for growing azolla which is covered with plastic gunnies to prevent the growth of roots of trees, soil temperature, and seepage water and later harvested with a scoop net with daily harvesting yield 10-12Kg/pit (Sonu Kumar Yadav .*et.al* 2023).

Azolla symbiotically associated cyanobacterium (Anabaena azollae). The cyanobacteria were isolated from Azolla pinnata and cultured under a free-living state. It is

observed filamentous brown to olive in color culture, its vegetative cells are cylindrical and their diameters ranged from 8 to 9µm width and from 5 to 6 µm length. Heterocysts were found to be internal and terminal in the filament, its shape is spherical and its diameters ranged from 9 to 9.5 µm width and from 9 to 9.5 µm length. Akintes are present with cylindrical shape, with diameters of 9-10 µm width and 6-8 µm length. Nitrogenase activity of Anabaena azollae increased gradually from 0.1786 to 4.7992 µ mole C_2H_4 g soil/hr along 4 weeks. The isolates also showed high efficacy in suppressing the fungal mycelial growth of either *Fusarium oxysporum or Alternaria*. (Azz A.M.Abd EI-AAI 2013).

In Egypt to study the vegetative growth, yield and fruit quality foliar spray with in aqueous solutions of biofertilizer (azolla and blue green algae extract at the rate of 1 L/20L water for each, commercial liquid organic fertilizer of humic acid (H.A.) at the rate of 200 cm3 /20 L water and their combinations were used. Trees of apricot (Prunus armeniaca L.) cv. Canino at 10- years-old trees grown in clay loam soil at 3 x 4 m apart under flood irrigation system. The obtained results indicated that most of the used treatments improved the means of blade width and length, leaf surface area, weight and total chlorophyll. Also, fruit set %, yield/tree, the fruit dimensions, volume and weight, as well as stone weight and flesh weight % take various significance levels as compared to control in both seasons. The present study recommended to spray the foliage of 10-years-old trees "Canino" apricot cv. grown under clay loamy soil with the aqueous extract Azolla + Blue green algae + humic acid, to increase both yield, fruit quality parameters and fruit storability (Nevine M. Taha1 and R. M. El-Shahat ,2017).

In the Krishi Vigyan Kendra, West Sikkim scientist were cultivate the azolla in the district conducted at farmers' field. The farmers were trend by on and off campus practical training cum demonstration on azolla production technology. The demonstration of azolla production unit was established by KVK Scientists and On Farm Trail (OFT) and Front Line Demonstration (FLD) on effect of fresh azolla as a nutrient supplementation to milch Cattle. For this purpose 30 lactating cattle of 2nd and 3rd lactation of 5-8 years old at mid lactation were selected for study and randomly distributed into two groups. The control (C) group (n=15) farmers practice was fed, paddy straw, green fodder, ground maize cooked with mustard oil cake whereas treatment (T) group (n=15) was fed same quantity of roughage, concentrate along with 1.5kg fresh Azolla/cattle/day. The feeding trial lasted for three month.

During trial daily milk yield, fat% and physical health status of animal were recorded and found the milk yield and fat percentage was significantly higher in treatment group. (Lhaki Doma Bhutia, TseringNorbu Bhutia *et al*2020).

It is observed that feed supplemented with azolla gives higher yield compare to the commercial feed. The study was conducted to know the effect of using Azolla (Azolla pinnata) as feed ingredient in dairy cows. The Azolla (Azolla pinnata) was cultivated by farmer in different sized plastic tubs in water. Animals were divided randomly into two equal groups; one group was fed on commercial concentrate feed (SKM) without Azolla supplementation and another group was fed with Azolla in commercial concentrate feed (SKM) at the ratio of 1:1 (1.5 kg Azolla + 1.5 kg commercial concentrate feed (SKM). All the animals were monitored daily for milk yield during the period of supplementation. The animals were maintained under proper managemental conditions. Animals were duly vaccinated and dewormed. Animals were milked twice daily (5 am and 5 pm) by full hand milking. Milk yield was measured by milk measuring pails. Azolla fed group showed a significant ($P<0.005$) increase up to 10.43% in milk production. (P.Suresh Kumar.2021).

Fig: Azolla production and its application

RESEARCH OBJECTIVES:

1. Isolation, Cultivation and Nutritional analysis of edible Azolla culture.
2. Isolation and characterization of endophytic microorganisms from Azolla
3. Biochemical characterization of isolated microbial cultures by culture dependent and independent method.
4. Analyze the impact of Azolla-Microbial consortium against milch animals: A field study.
5. Assessment of Milk yield and quality by analyzing its protein, fats and other contents.
6. Study of gut microbial load after effective implication of Azolla microbial consortium against the milch animal.
7. Development of Azolla pilot plant.
8. Technology dissemination and farmer training.
9. Thesis writing and submission.

METHODOLOGY AND PROPOSED WORK PLAN TO BE ADOPTED

Year 1: Foundational Work

1. Isolation, Cultivation, and Nutritional Analysis of Edible Azolla Culture.

Methodology:

- **Collection and Cultivation:** Collect Azolla species from KVK Chhatrapati Sambhaji nagar. Sterilize samples to remove contaminants and cultivate them in controlled conditions using nutrient-rich media.
- **Nutritional Analysis:** Conduct proximate analysis (moisture, protein, fat, and fiber content) using spectrophotometry and other methods.
- **Optimization:** Experiment with varying environmental conditions (seasonal variation) to enhance Azolla growth and nutritional content.

Significance:

- Establishes Azolla as a sustainable, cost-effective, and nutrient-rich feed supplement for milch animals.

2. Isolation and Characterization of Endophytic Microorganisms from Azolla.

Methodology:

- **Isolation:** Sterilize Azolla tissues and extract endophytes using dilution plating on selective nutrient media (e.g., nitrogen-free media for diazotrophs, PDA for fungi).
- **Characterization:** Conduct morphological and biochemical tests (Gram staining, enzymatic assays).

Significance:

- Identifies beneficial microorganisms that can improve Azolla's growth, nutrient uptake, and digestibility.

Year 2: Functional Analysis and Consortium Development

3. Biochemical Characterization of Isolated Microbial Cultures (Culture-Dependent and Independent Methods).

Methodology:

- **Culture-Dependent:** Perform biochemical assays to identify microbial traits like nitrogen fixation, phosphate solubilization, cellulase activity etc.
- **Culture-Independent:** Use 16S rRNA sequencing to identify the isolate.

Significance:

- Provides a comprehensive understanding of microbial functionality.
- Enhances the nutritional profile and digestibility of Azolla through microbial inoculation.

4. Analyze the Impact of Azolla-Microbial Consortium against Milch Animals

Methodology:

- **Consortium Development:** Combine Azolla with selected microbial strains optimized for nitrogen fixation, nutrient enhancement etc.
- **Feeding Trials:** Conduct feeding experiments on milch animals by dividing them into control and test groups (traditional feed, Azolla alone, Azolla-microbial consortium). Monitor feed intake, body weight, and general health.

Significance:

- Validates the Azolla-microbial consortium's effectiveness in improving the health and productivity of milch animals.
- Establishes the feasibility of integrating Azolla-microbial consortia into dairy farming systems.

Year 3: Application and Validation:

5. Assessment of Milk Yield and Quality by Analyzing Its Protein, Fat, and Other Contents.

Methodology:

- **Milk Analysis:** Collect milk samples from feeding trials and analyze quality parameters (protein, fat, lactose, total solids and other) using standard methods.

Significance:

- Demonstrates the Azolla-microbial consortium's impact on milk quality and nutritional value.
- Highlights the economic benefits of improved milk yield and quality for farmers.

6. Study of Gut Microbial Load after Effective Implication of Azolla-Microbial Consortium against Milch Animals.

Methodology:

- **Sample Collection:** Collect fecal samples from control and test groups.
- **Microbial Analysis:** Perform biochemical analysis of gut microbiota to identify the bacterial diversity and abundance.
- **Health Markers:** Assess gut health indicators like enzyme activity.

Significance:

- Provides insights into gut health improvements due to Azolla-microbial consortia.
- Establishes the correlation between gut microbiota and milk productivity.

7. Development of Azolla Pilot Plant:

Methodology:

- **Design and Setup:** Build a small-scale Azolla production unit with integrated microbial inoculation capabilities. Incorporate automation for water, light, and nutrient management.
- **Scalability Testing:** Produce sufficient quantities of Azolla-microbial consortia for extended field trials and economic feasibility studies.

Significance:

- Demonstrates the scalability of Azolla production for widespread adoption.
- Facilitates technology transfer to farmers for sustainable dairy farming.

1. **Technology Dissemination and Farmer Training.**

Methodology:

- Develop farmer-friendly protocols for Azolla cultivation, microbial consortium preparation, and feed usage.
- Conduct training sessions and on-site demonstrations to promote the technology among dairy farmers.

Significance:

- Ensures technology adoption for improving dairy farming practices.
- Empowers farmers with sustainable, cost-effective solutions for enhancing milk production.

IMPORTANCE OF STUDY/ SOCIETY APPLICATION:

1. **Sustainable Dairy Farming**: The use of *Azolla*-microbial consortia provides a low-cost, eco-friendly feed supplement that reduces the reliance on expensive commercial feeds, thereby promoting sustainable dairy farming practices.
2. **Improved Milk Yield and Quality:** Enhanced milk production with better protein, fat, and nutritional content benefits farmers by increasing their income and provides consumers with high-quality dairy products.
3. **Reduction in Feed Costs**: Cultivation of *Azolla* as a locally available feed resource significantly lowers the cost of feeding milch animals, making dairy farming economically viable for small and marginal farmers.
4. **Environmental Benefits***: Azolla* cultivation absorbs carbon dioxide and releases oxygen, contributing to carbon sequestration and improving local water quality by reducing nutrient pollution in aquatic ecosystems.
5. **Enhanced Animal Health**: The probiotics and nutrients from the microbial consortium improve gut health in animals, leading to better digestion, reduced disease incidence, and enhanced overall health.
6. **Reduction of Antibiotic Use**: Improved gut health and immunity in animals through the *Azolla*-microbial consortium reduce the need for antibiotics, minimizing the risk of antibiotic residues in milk and the emergence of antimicrobial resistance.
7. **Empowerment of Rural Farmers**: The adoption of this technology provides rural farmers with a sustainable, accessible, and low-cost feed option, empowering them to increase productivity and profitability.

8. **Nutritional Security for Consumers**: By improving the quality of milk with higher protein and fat content, this research contributes to better nutritional outcomes for the general population, addressing malnutrition concerns.
9. **Efficient Resource Utilization**: *Azolla* requires minimal land, water, and i14nputs for cultivation, making it a resource-efficient feed supplement suitable for regions with limited agricultural resources.
10. **Technology Transfer and Knowledge Sharing**: Through farmer training and pilot plant development, this research facilitates the dissemination of practical knowledge and innovative farming techniques to the agricultural community.
11. **Mitigation of Climate Change Impacts**: By reducing the dependency on resource-intensive commercial feed production, this initiative contributes to reducing greenhouse gas emissions associated with traditional dairy farming.
12. **Promotion of Circular Economy**: The integrated use of *Azolla* and microbial consortia aligns with circular economy principles by utilizing natural resources efficiently, improving waste management, and promoting sustainable agricultural practices.

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